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ORIGINAL INVESTIGATION

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Location of the gene causing hyperimmunoglobulinemia D and periodic fever syndrome differs from that for familial mediterranean fever

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Abstract The hyperimmunoglobulinemia D and periodic fever (hyper-IgD) syndrome is typified by recurrent febrile attacks with abdominal distress, joint involvement (arthralgias/arthritis), headache, skin lesions, and an elevated serum IgD level (>100 U/ml). This familial disorder has been diagnosed in 56 subjects worldwide. As the hyper-IgD syndrome resembles familial Mediterranean fever, one could speculate that both result from mutations in the same gene. The gene causing familial Mediterranean fever (MEF) has been located on chromosome 16p. We have studied 10 families with 19 affected and 28 non-affected subjects. The clinical findings and IgD determinations from these families are compatible with autosomal recessive inheritance. Using highly polymorphic markers surrounding the MEF gene, only negative Lod scores were obtained, whereas haplotype analysis excluded this locus as the cause of the hyper-IgD syndrome. In addition, no indication for linkage was obtained with markers from other candidate gene regions on chromosomes 17q and 14q.

Introduction

The hyperimmunoglobulinemia D and periodic fever syndrome (hyper-IgD) is a rare entity characterized by recurrent attacks of fever (Van der Meer et al. 1984). Symptoms start at an early age and persist throughout life (Drenth et al. 1994a). Diagnosis is made by using clinical criteria allowing distinction from other periodic fever syndromes, such as familial Mediterranean fever (FMF), adult-onset Still's disease, and juvenile chronic arthritis (Drenth et al. 1994a). Typically, the attacks occur every 4–8 weeks and last 3–7 days; they are accompanied by abdominal distress (vomiting, diarrhoea, and pain), joint involvement (arthralgia/arthritis), skin lesions, headache, and lymphadenopathy (Drenth et al. 1994a, b; Van der Meer et al. 1984). All patients have a persistently elevated polyclonal serum IgD level (> 100 U/ml) (Hiemstra et al. 1989). The pathogenesis of the hyper-IgD syndrome remains an enigma and consequently therapy is solely supportive. So far, 56 patients (29 male/27 female) from nine countries have been diagnosed. Autosomal recessive inheritance is suggested by the finding that in seven families, two or more sibs are affected, whereas the parents are unaffected. Serum IgD measurements in one family revealed high values in patients and low values in unaffected members (<100 U/ml), supporting the concept of autosomal recessive inheritance (Reeves and Mitchell 1984).

The febrile attacks of the hyper-IgD syndrome and FMF have much in common, despite some clinical differences, such as lymphadenopathy (rare in FMF), serositis (rare in hyper-IgD syndrome), and amyloidosis (not seen in hyper-IgD syndrome). Both syndromes are typified by recurrent abdominal distress, articular symptoms, and skin manifestations.

There are also similarities in the biochemical changes that accompany attacks, namely, high erythrocyte sedimentation rate, leukocytosis, and high levels of C-reactive protein (Drenth et al. 1994a; Meyerhoff 1980). In view of these similarities, it could be speculated that FMF and the hyper-IgD syndrome are variants of one condition (Federspiel and Tonz 1987; Majeed and Barakat 1989), and that both syndromes are allelic and produced by defects in the same gene. Initially, in Israeli families of North African and Iraqi (non-Askhenazi) descent, linkage was indicated between FMF and markers on chromosome 17q22-q24 (Aksentijevich et al. 1993a). A maximum multipoint Lod score of 3.27 was reached approximately 10 centimorgans (cM) telomeric to D17S40. Subsequent linkage studies

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have however failed to support chromosome 17q as the location for the responsible gene (Aksentijevich et al. 1993a). In the same non-Askhenazi Israeli families, the gene causing FMF (the MEF gene) could be mapped to the short arm of chromosome 16 (Pras et al. 1992). Using marker D16S84 (16p13.3), a lod score of 9.17 was reached at a recombination frequency (θ) of 0.04. These results also pertain to Armenian families suffering from FMF (Shohat et al. 1992). Refined mapping with highly polymorphic markers resulted in the localization of the MEF gene less than 1 cM centromeric from D16S246 (Aksentijevich et al. 1993b; Levy et al. 1993). Such accurate localization allows us to examine the role of the MEF gene in the hyper-IgD syndrome.

Materials and methods

Patients

The present study included 10 families with members suffering from the hyper-IgD syndrome (Fig. 1). Seven families originate from the Netherlands (nos. 2, 5, 8–10), the other three families are from the United Kingdom (no. 6), France (no. 1), and Spain (no. 7), respectively. There were no consanguineous marriages. In total, 19 affected and 28 non-affected family members were investigated. The clinical data pertaining to these patients have been published elsewhere and the diagnosis of hyper-IgD syndrome was made according to published criteria (Drenth et al. 1994a). Briefly, the patients suffer from life-long recurrent self-limiting attacks of fever, with no known antigenic triggers, lasting 3–7 days and accompanied by one or a combination of the following symptoms: abdominal distress (vomiting, diarrhea, pain), skin manifestations, arthralgia/arthritis, and/or lymphadenopathy. A detailed family history and pedigree were obtained by interviewing each patient; a comprehensive medical history was also taken from each unaffected family member. Immunoglobulin D concentrations were measured by enzyme-linked immunosorbent assay with a detection limit of 1 U/ml. The study was approved by the Medical Ethics Committee of the University Hospital St Radboud, Nijmegen.

DNA analysis

Blood was sampled from relevant family members, and genomic DNA was isolated according to the method of Miller et al. (1988). The markers studied were D16S418, D16S423, D16S283, and D16S291 (chromosome 16p13.3), D17S515 (chromosome 17q22-24), and D14S78 (chromosome 14q32-33).

All markers were analyzed using polymerase chain reaction amplification of genomic DNA (50 ng) in a 15-ml reaction mixture containing 200 μM of each dATP, dGTP, dTTP, 2.5 μM dCTP, 0.6 μCi ^{32}P-dCTP (10 mCi/ml, 3000 Ci/mmol), 1 × Supertaq buffer [10 mM TRIS-HCl pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.01% (w/v) gelatin] and 0.06 U Supertaq (HT Biotechnology, United Kingdom) overlayed with mineral oil. After an ini-

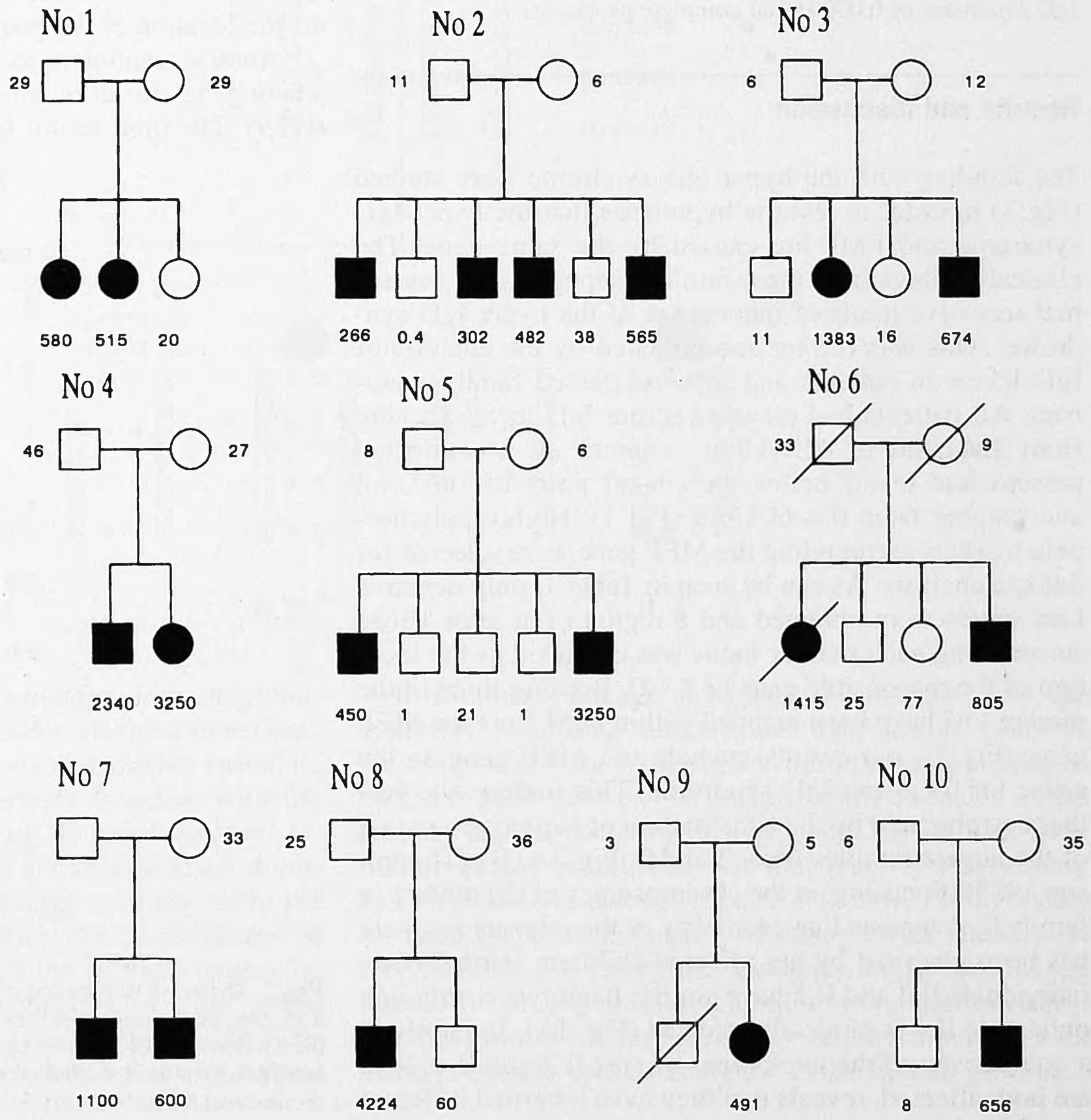

Fig. 1 Ten hyper-IgD families studied for linkage analysis with polymorphic markers. In total, 19 affected patients and 33 non-affected family members were present. Results of IgD measurements in U/ml are shown for each individual. Note the high serum IgD concentrations in patients (>100 U/ml) and the low values for unaffected individuals (<100 U/ml)

Table 1 Linkage analysis between the gene for hyper-IgD syndrome and markers of candidate regions for the gene

Marker	Location	Lodscores θ						
		0.00	0.01	0.05	0.1	0.2	0.3	0.4
D16S291	16q13.3	$-\infty$	–5.64	–2.36	–1.16	–0.31	–0.07	–0.01
D16S283	16q13.3	$-\infty$	–5.08	–2.43	–1.40	–0.54	–0.18	–0.04
D16S423	16q13.3	$-\infty$	–6.58	–2.72	–1.35	–0.38	–0.09	–0.01
D16S418	16q13.3	$-\infty$	–7.09	–3.17	–1.70	–0.56	–0.16	–0.03
D17S515	17q22	$-\infty$	–10.6	–5.19	–3.02	–1.18	–0.41	–0.08
D14S78	14q32.3	$-\infty$	–4.21	–1.62	–0.68	–0.04	–0.07	0.03

tial denaturation for 5 min at 94°C, amplification was performed with 30 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 1 min, followed by a final extension of 6 min at 72°C (Weber and May 1989). The reactions were performed in a 96-well thermal cycler (M.J. Research, Watertown, Mass, USA). After amplification, samples were analyzed on a 6.6% polyacrylamide gel (acrylamide: N,N`-methylene bisacrylamide 19:1) containing 8.3 *M* urea, 1 × TBE (100 m*M* TRIS-borate, 2 m*M* EDTA pH 8.3). Electrophoresis was performed at 60 W for 2–4 h. After drying of the gels, bands were visualized by overnight exposure to Kodak X-OMAT S film.

Statistical analysis

Linkage analysis was performed using the MLINK subroutine of the computer program LINKAGE (version 5.10) (Lathrop et al. 1985). Lod score calculations were performed on the basis of autosomal recessive inheritance with a gene frequency for the hyper-IgD syndrome of 0.00001 and complete penetrance.

Results and discussion

Ten families with the hyper-IgD syndrome were studied (Fig. 1) in order to test the hypothesis that the hyper-IgD syndrome and FMF are caused by the same gene. The clinical findings from these families supported the autosomal recessive mode of inheritance of the hyper-IgD syndrome. This was further substantiated by the analysis of IgD levels in patients and in non-affected family members. All patients had elevated serum IgD levels varying from 266 U/ml to 4224 U/ml, whereas all non-affected persons had levels below the cut-off point of 100 U/ml and ranging from 0.4–60 U/ml (Fig. 1). Highly polymorphic markers surrounding the MEF gene were selected for linkage analysis. As can be seen in Table 1, only negative Lod scores were obtained and a region of at least 10 cM surrounding each marker locus was excluded as the location of the responsible gene ($Z \leq -2$). Because three of the marker loci have been mapped within 5 cM from the MEF gene (Fig. 2), our results exclude the MEF gene as the cause for the hyper-IgD syndrome. This finding was further corroborated by the construction of haplotypes in two of the largest families (nos. 2 and 3; Fig. 3A, B) (Thompson 1987). Focusing on the chromosomes of the mother in family 2, it appears that each copy of the relevant segment has been inherited by her affected children. Furthermore, individuals II.4 and II.5 have similar haplotypes, although only male II.4 is clinically affected (Fig. 3A). In family 3, a comparison of the haplotypes of sibs II.2 and II.4, who are both affected, reveals that they have inherited different combinations of parental segments of 16p (Fig. 3B). These findings exclude the MEF gene as the cause for hyper-IgD syndrome in these families. After the exclusion of the MEF gene, we decided to test two other candidate regions.

Markers from the region 17q22-q24 have previously shown linkage with the disorder in a subset of MEF families (Aksentijevich et al. 1993a). Multi-point linkage analysis indicates that the most likely position of the gene was at 10 cM telomeric to D17S40. However, linkage analysis in our families with the marker D17S515, which has been mapped at this location (NIH/CEPH collaborative mapping group 1992), resulted in a negative lod score (Table 1). A region of about 30 cM surrounding this marker locus was excluded; this argues against the 17q region as the location of the gene for hyper-IgD syndrome.

Another candidate locus is the immunoglobulin heavy chain gene cluster on chromosome 14q32.3 (Hofker et al. 1989). The high serum IgD concentration persists during

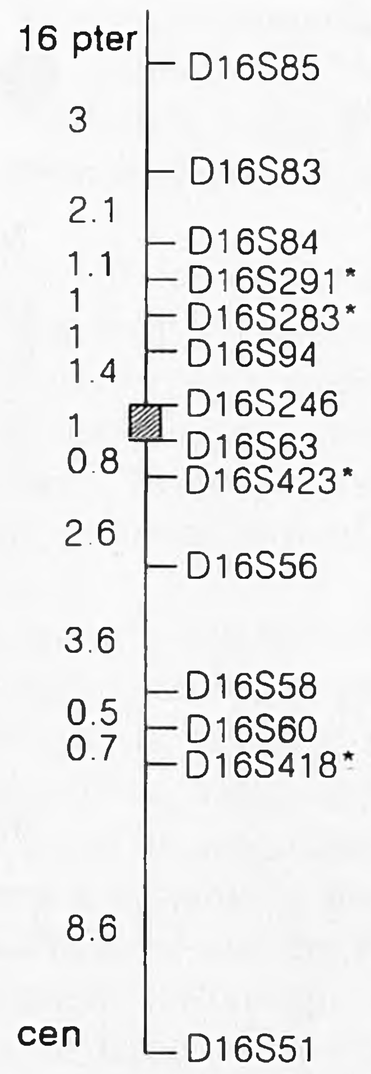

Fig. 2 Order of polymorphic markers surrounding the MEF gene. The genetic distances (sex averaged) are given in cM according to the Genome Data Base (Aksentijevich et al. 1993b). The *hatched box* indicates the location of the MEF gene. The *asterisks* point to the microsatellite markers examined in the current study

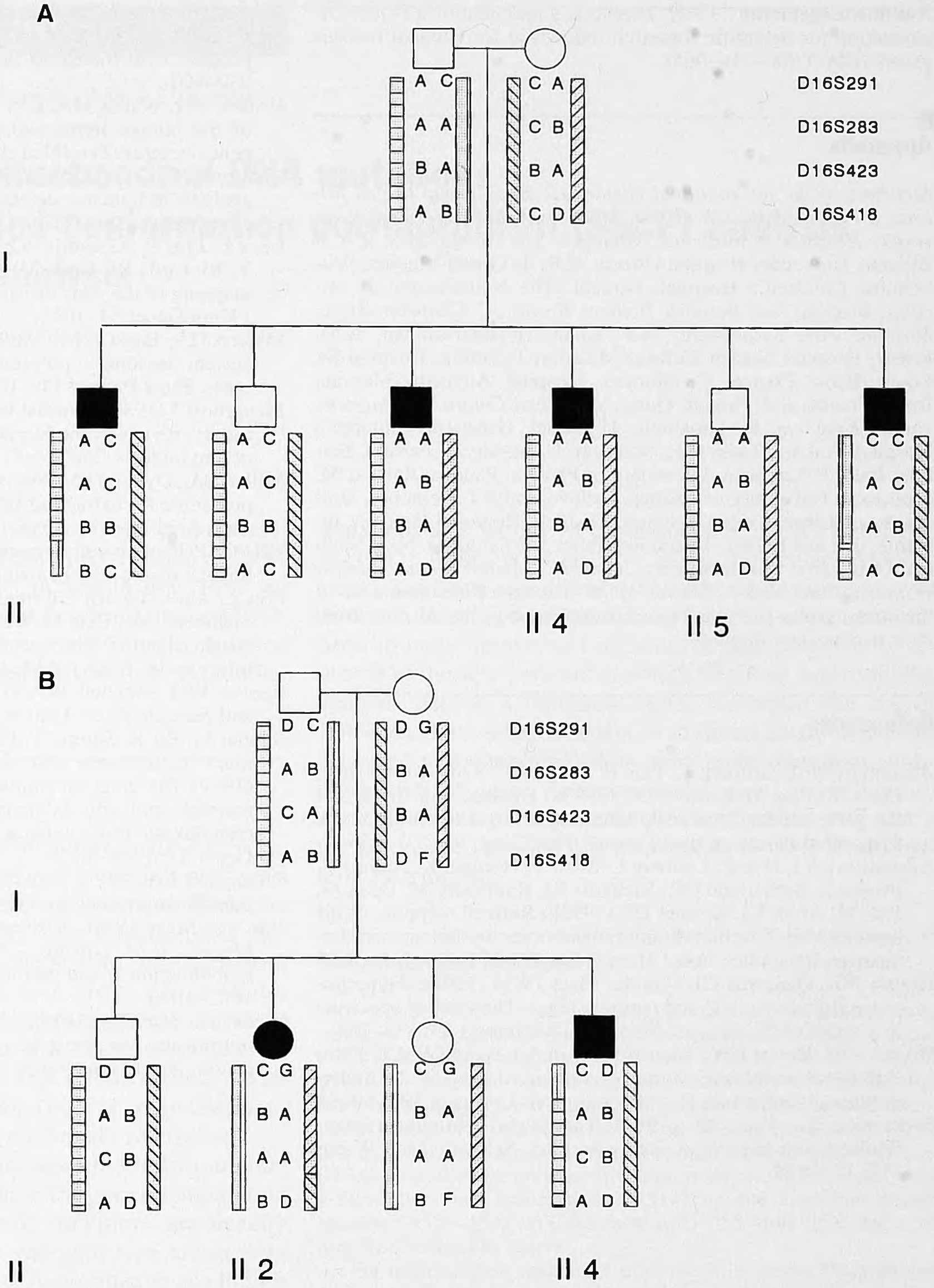

Fig. 3 Haplotype analysis of the relevant area of chromosome 16p for family 2 (**A**) and family 3 (**B**). The orientation of the markers is 16pter-D16S291-D16S283-D16S423-D16S418-16cen. Haplotypes were assigned in such a way as to minimize the number of crossovers in each family (Thompson 1987)

non-symptomatic periods, and other serum immunoglobulins such as IgA and IgG_3 can also be elevated in patients with hyper-IgD syndrome. One could therefore speculate that a mutation at the Ig-locus might interfere with the regulation of several members of the gene cluster. Although such a mutation would be anticipated to act dominantly, we have performed linkage analysis with the marker D14S78, which is located an estimated distance of 8 cM from the Ig-locus. Although the Ig-locus cannot be firmly excluded (Table 1), the negative Lod score would not support the presence of a mutation in the immunoglobulin heavy chain gene cluster causing hyper-IgD syndrome.

In summary, linkage analysis in 10 families with the hyper-IgD syndrome demonstrates that, despite many of the symptoms of the hyper-IgD syndrome being similar to FMF, the MEF gene can be unequivocally excluded as the primary disease locus for the hyper-IgD syndrome. This finding firmly establishes that the hyper-IgD syndrome and FMF are two distinct genetic disorders. Furthermore, we have found no indication of linkage with markers on chromosome 17q (foregoing evidence suggested linkage with FMF) or 14q (immunoglobulin heavy chain cluster). The localization of the gene may require a search with markers equally spaced along the entire human genome.

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Appendix

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